Journal of the Lepidopterists' Society
45(3), 1991, 215–221

OVIPOSITION BY *DANAUS PLEXIPPUS* (NYMPHALIDAE: DANAINAE) ON *ASCLEPIAS VIRIDIS* IN NORTHERN FLORIDA

TONYA VAN HOOK
Department of Entomology and Nematology, University of Florida,
Gainesville, Florida 32601

AND

MYRON P. ZALUCKI
Department of Entomology, The University of Queensland,
Brisbane, Queensland, Australia 4072

ABSTRACT. Female monarch butterflies, *Danaus plexippus* L. (Danainae), remigrating in spring from overwintering sites in Mexico encounter and oviposit on three species of *Asclepias*, *A. humistrata* Walt., *A. asperula capricornu* (Woods.) Woods., and *A. viridis* Walt. (Asclepiadaceae), in the southeastern U.S.A. These plants are relatively high, but quite variable, in gross cardiac glycoside (CG) concentration, both within and among species. We compared CG concentration between plants in a single stand of *A. viridis* with and without immatures (eggs and larvae) of *D. plexippus*. Plants with immatures showed an intermediate CG concentration (280 μg/0.1 g DW), with lower variability (SD = 104, N = 20), than plants without immatures (314 ± 176, N = 10). This study supports previous findings that females oviposit preferentially on plants with intermediate CG content in single species stands. Other studies indicate that it is on these plants that sequestration by larvae of CGs is maximal.

Additional key words: Asclepiadaceae, cardiac glycosides, immature distribution.

Monarch butterflies, *Danaus plexippus* L. (Nymphalidae: Danainae), remigrating from overwintering sites in Mexico in early spring encounter and oviposit on primarily three species of *Asclepias* in the southeastern U.S.A. These species, A. *humistrata, A. viridis,* and *A. asperula capricornu,* all have relatively high concentrations of cardenolides, or cardiac glycosides (CG) (Malcolm & Brower 1989).

Monarchs breed in the southeastern United States for one to two generations before high temperatures apparently force them to continue migrating northward (Malcolm et al. 1987, 1991). One consequence of this northward colonization is the use of a larval host, *A. syriaca* L., that is relatively low and variable in CG concentration. Because monarchs sequester CG's, which make them unpalatable to potential predators, they would be better protected if they utilized plants higher in CG's. Thus butterflies feeding as larvae on *A. syriaca* may be relatively palatable (Malcolm et al. 1988).

The relationship between female oviposition site selection to the CG content of the larval food plant is unclear (see Oyeyele & Zalucki 1990, for a review). Brower (1961) found more monarch immatures on *A. humistrata,* which is high in CG content, than on *A. tuberosa rolfsii*

(Britton) Woodson, which is very low in CG content, at a site where the two plant species co-occur. However, direct observation of ovipositing monarchs in two very different populations (on A. *fruticosa* L. in Australia and A. *humistrata* in northern Florida) showed that females selected plants of intermediate CG concentration (Oyeyele & Zalucki 1990, Zalucki et al. 1990). In both studies females showed post-alighting discrimination against plants with very low and those with very high CG concentrations.

In this study, we examined the distribution of immature monarchs on another species of milkweed, A. *viridis*. We analyzed the CG concentration of plants with and without immature monarchs to determine whether females were discriminating among plants based on their CG concentration.

MATERIALS AND METHODS

We sampled 30 A. *viridis* plants in a limestone outcropping adjacent to a quarry near Williston, Levy Co. (29°25'N, 82°28'W), in north central Florida on 16 April 1987. All plants were within 20 m of each other, on the northern, eastern, and southern margins of an old quarry. All plants stood out from a low undergrowth of grasses. The stems of all plants sampled were counted, their heights measured and averaged, and each plant's phenological stage was classified using a 3 point scale as flowering (=3), with immature flower buds (=2), not yet flowering (=1). All monarch eggs and larvae were counted and their location on the plants noted. Leaf samples from the same position on all plants (third leaf pair from the top of the northern most stem), were placed in separate labelled bags for CG analysis. We attempted to obtain equal numbers of plants in three categories: (1) without immature monarchs, (2) with eggs only, and (3) with eggs and larvae. We took flower material and leaf material for comparative CG analysis from 4 plants that were in flower and had eggs on them. All plant material was dried for 16 h at 60°C in a forced draft oven and stored in labelled envelopes inside a desiccator until processing. Cardiac glycoside determinations were done within two weeks of collection using a standard spectrophotometric technique (Brower et al. 1975, 1984).

RESULTS AND DISCUSSION

Mean plant phenological stage, size, number of monarch immatures, and cardiac glycoside concentrations are listed in Table 1 for plants in 3 categories: without monarchs, with eggs only, and with eggs and larvae. All but 2 of 31 plants were either flowering (n = 14) or with buds (n = 15). The average plant phenological stage (using a 3 point scale for stage categories) was similar in plants without and with mon-

TABLE 1. Summary of observations made on *Asclepias viridis* at Williston, Florida, showing mean phenological stage (see text for details), size (average height, multiplied by the number of stems), eggs and larvae per plant, and plant CG concentration. Plants have been grouped as without immature monarchs, with eggs only, and with eggs and larvae. (Values in brackets are standard deviations.)

	Plant monarch status		
Parameter	Without monarchs	Eggs only	Eggs & larvae
N	11*	9	11
Phenological stage	2.2 [0.75]	2.8 [0.44]	2.8 [0.40]
Size (cm)	73 [42.6]	255 [150.7]	257 [201.3]
Eggs	—	1.2	1.8
Larvae	—	—	2.7
CG concentration (μg/0.1 g DW)	314 [176]	265 [127]	294 [86]

* One plant sample lost for CG analysis.

archs (Table 1). Plants with eggs only, and those with eggs and larvae, did not differ in size (Table 1), but those without eggs were markedly smaller than the rest ($P < 0.05$, t-test). Among plants with eggs only, eggs were distributed evenly; 7 plants had 1 egg each and 2 had 2 eggs. Plants with eggs and larvae had the following dispersion: one plant had 9 eggs and 4 larvae, another had 1 egg and 8 larvae. The remaining plants had (in sampling order): 0, 3, 0, 0, 2, 0, 1, 0, and 2 eggs, and a corresponding larval distribution of: 1, 1, 1, 1, 3, 1, 3, 2, 2. Larvae ranged in age from first (I) to third (III) instar with the following age distribution: 6I, 13II, and 8III.

There was a strong correlation between eggs, larvae, and total immatures per plant and plant size ($r = 0.73$, 0.45, and 0.71, $N = 30$, $P < 0.05$, respectively). There was no relationship between plant size (number of stems multiplied by the average height) and CG concentration ($r = 0.14$, $P > 0.05$). These results are very similar to those obtained by Cohen and Brower (1982) for a small sample ($N = 9$) of *A. humistrata*. However, it is difficult to infer a relationship between oviposition and larval food plant CG levels based on the pattern of distribution of immatures in the absence of direct observations of oviposition (Zalucki et al. 1989).

Based on observational data, Oyeyele and Zalucki (1990) and Zalucki et al. (1990) found that females were more likely to lay eggs on plants of intermediate CG concentration and reject lower and higher CG concentration plants. Therefore we predict that the CG frequency distribution of plants with immatures should have a smaller variance than plants without immatures. In making this prediction we are assuming that: (1) CG levels do not change rapidly in response to larval feeding, and (2) that all plants without immatures could have been sampled by adults. The latter seems reasonable as all plants were exposed, relatively

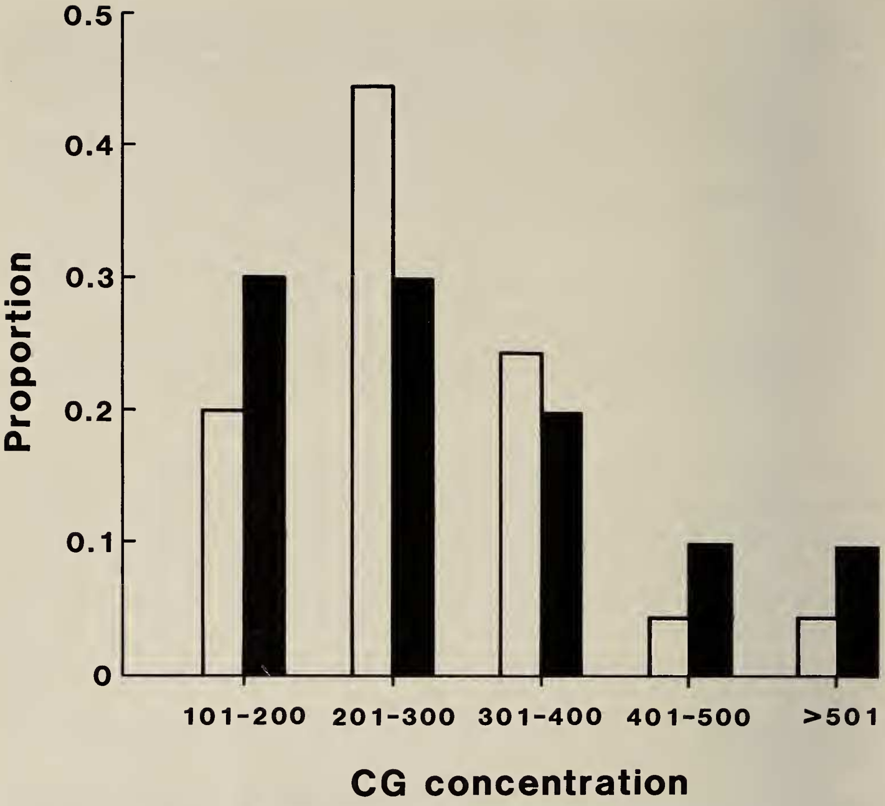

FIG. 1. Distribution of plant cardiac glycosides expressed as ranges of CG concentration (μg/0.1 g DW), in plants without (solid bars) and with (open) immature monarchs ($\bar{x}$ without = 314; $\bar{x}$ with = 280 μg/0.1 g DW).

close to each other (ca. 0.5 to 1.5 m apart), and our sampling included virtually all (ca. 80%) of the plants in the area.

Although the mean cardenolide content was lower in plants with eggs and immatures than those without (Table 1), the differences were not significant ($P > 0.05$). However, the distribution of CG concentration among plants with immatures was less variable than among plants without (F ratio test, $F_{10,20} = 2.863$, $P < 0.05$).

The frequency distribution of CG's in plants with immatures is contained within the distribution of plants without immatures (Fig. 1), with most immatures (70%) being found on plants with CG concentrations in the range 201–400 μg/0.1 g dry weight (DW). This supports findings by Oyeyle and Zalucki (1990) and Zalucki et al. (1989, 1990) that females select plants of intermediate levels of CG concentration for oviposition.

TABLE 2. Cardiac glycoside (CG) concentrations (μg/0.1 g DW) recorded in *Ascelpias viridis* by various authors.

Location	CG concentration N	x̄	SD	Range	Reference
Florida	7	478	136	316–676	Malcolm and Brower 1986
Florida	18	376	203	148–972	Malcolm and Brower 1989
Louisiana	60	245	70	95–432	Lynch and Martin 1987
Florida	30	292	130	106–730	This study

Many eggs (19 out of 29) were found on the inflorescenses of *A. viridis.* We measured CG concentration in leaves and inflorescenses from the same plant. For 4 plants with immatures the inflorescenses had an average CG level of only 42% of the leaf sample readings. The CG values (in μg/0.1 g DW) for leaf vs. flower samples for each plant were: 332 vs. 116, 206 vs. 16, 106 vs. 88, and 159 vs. 67. This suggests that monarchs are ovipositing on plant parts with a lower CG concentration. Similar observations were made on *A. linaria* Cav. in Mexico, a very high CG plant (1400 μg/0.1 g DW); immatures were found on plants, and on plant parts (including inflorescenses), with the lowest CG concentrations (L. P. Brower & M. P. Zalucki, unpubl. data). However, it should be noted that the inflorescences of *A. viridis* are also much softer than the leaves, and females may be assessing the 'quality' of plant parts on the basis of texture rather than on CG concentration.

Our results for CG concentration in *A. viridis* are within the range of those published by Lynch and Martin (1987) and Malcolm and Brower (1986, 1989) (Table 2). Although *A. viridis* is generally a high CG plant, the range in CG concentration (95–972 μg/0.1 g DW) is considerable (Table 2). Such variation could be due to many factors, including plant part sampled, seasonal effects, and method of CG determination between labs. Because we sampled leaves from the same position on each plant, differences between plants presumably must be due to differences primarily in genotype, age, phenological stage, and localized soil and moisture conditions. Our results indicate that the pattern of occurrence of immature monarchs on *A. viridis* plants is similar to those found in more extensive studies on other milkweed species (Oyeyele & Zalucki 1990, Zalucki et al. 1989, 1990). Namely, plants in the intermediate CG concentration category are more likely to support monarchs than are plants with either very low or very high CG concentrations.

The plants on which immatures are found have CG concentrations at which sequestration is maximal and the resultant adults have the highest CG level obtainable, given the logarithmic relationship between plant CG and the adult CG level (Malcolm & Brower 1989, Nelson 1991). To demonstrate an oviposition preference based on CG concentration, further work on *A. viridis* is needed, in which ovipositing females are followed and plants accepted and rejected are assayed for CG concentration.

ACKNOWLEDGMENTS

We thank Lincoln Brower for providing facilities and for constructive comments on the manuscript, Steve Malcolm for his encouragement, and Jocelyn Campbell for typing. This research was supported by NSF grant # BSR-8500416, with L. P. Brower as Principal Investigator. M. P. Zalucki was on study leave from the University of Queensland.

LITERATURE CITED

BROWER, L. P. 1961. Studies on the migration of the monarch butterfly I. Breeding populations of *Danaus plexippus* and *D. gilippus berenice* in south central Florida. Ecology 43:549–552.

BROWER, L. P., M. EDMONDS & C. M. MOFFITT. 1975. Cardenolide content and palatability of a population of *Danaus chrysippus* butterflies from West Africa. J. Entomol. 49:183–196.

BROWER, L. P., J. N. SEIBER, C. J. NELSON, S. P. LYNCH, M. P. HOGARD & J. A. COHEN. 1984. Plant determined variation in cardenolide content and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus* reared on milkweed plants in California, 3: *Asclepias californica*. J. Chem. Ecol. 10:1823–1857.

COHEN, J. A. & L. P. BROWER. 1982. Oviposition and larval success of wild monarch butterflies (Lepidoptera: Danaidae) in relation to host plant size and cardenolide concentration. J. Kans. Entomol. Soc. 55:343–348.

LYNCH, S. P. & R. A. MARTIN. 1987. Cardenolide content and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus* L., and their larval host-plant milkweed, *Asclepias viridis* Walt., in northwestern Louisiana. J. Chem. Ecol. 13:47–70.

MALCOLM, S. B. & L. P. BROWER. 1986. Selective oviposition by monarch butterflies *Danaus plexippus* L. in a mixed stand of clepias curassavica L. and *A. incarnata* L. in South Florida. J. Lepid. Soc. 40:255–263.

MALCOLM S. B. & L. P. BROWER. 1989. Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. Experientia 45:284–295.

MALCOLM, S. B., B. J. COCKRELL & L. P. BROWER. 1987. Monarch butterfly voltinism; effects of temperature constraints at different latitudes. Oikos 49:77–82.

MALCOLM, S. B., B. J. COCKRELL & L. P. BROWER. 1988. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. J. Chem. Ecol. 15:819–853.

MALCOLM, S. B., B. J. COCKRELL & L. P. BROWER. 1991. Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration? *In* Malcolm, S. B., and M. P. Zalucki (eds.), The biology and conservation of the monarch butterfly. Natural History Museum of Los Angeles County, Contributions in Science, *in press*.

NELSON, C. 1991. A model for cardenolide and cardenolide glycoside storage by the monarch butterfly *Danaus plexippus* (L.). *In* Malcolm, S. B., and M. P. Zalucki (eds.), The biology and conservation of the monarch butterfly. Natural History Museum of Los Angeles County, Contributions in Science, *in press*.

OYEYELE, S. V. & M. P. ZALUCKI. 1990. Cardiac glycosides and oviposition by *Danaus plexippus* on *Asclepias fruticosa* in south-east Queensland (Australia), with notes on the effect of plant nitrogen content. Ecol. Entomol. 15:177–185.

ZALUCKI, M. P., OYEYELE, S. V. & P. VOWLES. 1989. Selective oviposition by *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) in a mixed stand of *Asclepias fruticosa* and *A. curassavica* in Southeast Queensland. J. Aust. Entomol. Soc. 28:141–146.
ZALUCKI, M. P., L. P. BROWER & S. B. MALCOLM. 1990. Oviposition by *Danaus plexippus* in relation to cardenolide content of three *Asclepias* species in the south-eastern U.S.A. Ecol. Entomol. 15:231–240.

Received for publication 17 July 1990; revised and accepted 17 July 1991.